Comparison of vineyard-associated Saccharomyces cerevisiae populations by microsatellite analysis

Dorit Schuller[1], Eva Valero[2,3], Brigitte Cambon[2], Sylvie Dequin[2], Margarida Casal[1]

[1] Departamento / Centro de Biologia, Universidade do Minho, Braga, Portugal
[2] UMR Sciences pour l'Oenologie, INRA, Montpellier, France
[3] Present address: Departamento de Agroalimentación, IMIDRA, Madrid, Spain

The aim of the present study was to evaluate populational structures among fermenting S. cerevisiae populations. Analysis of six polymorphic microsatellite loci was performed in 468 S. cerevisiae isolates derived from a previous screening (using mtDNA RFLP or electrophoretic karyotyoing) of 2490 yeast strains obtained from spontaneous fermentations of grapes collected in three vineyards of the Vinho Verde Region (northwest Portugal), and one vineyard of the Languedoc Region (South France) during the 2001 – 2003 harvest seasons. Among the 93 alleles obtained, 52 new alleles were identified. For all loci analyzed, observed heterozygosity was three to four times lower than the expected value, probably due to a strong populational substructuring. Populational structures were identified based on the accumulation of small allele-frequency differences across six loci in groups of strains. The present work is the first large-scale approach showing that microsatellite typing reveals a very fine population resolution of indigenous S. cerevisiae strains isolated from vineyards.

Introduction

The grape's yeast flora depends on a large variety of factors such as climatic conditions including temperature and rainfalls, the geographic localization of the vineyard, antifungal applications, the harvest technique, grape variety, the vineyard's age as well as the soil type. Several ecological surveys report a large diversity of Saccharomyces sp. strains among the enological fermentative flora. Some strains seem to be widely distributed in a given viticultural region, can be found in several consecutive years and are also predominant in the fermenting flora hypothesizing the occurrence of specific native strains that can be associated to a terroir [1-2].

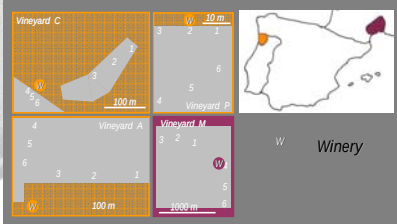
At present, leading winemakers demand for autochthonous fermenting strains that are able to enhance the expression of typical sensorial characteristics of wine and ensure the control of the fermentation process, concerning the motto "special yeasts for special traits" [3]. The detailed biogeographical evaluation of fermentative strains is essential for the establishment of adequate selection and improvement programes.

The aim of the present study was to gain insight in the populational structure of vineyard-associated S. cerevisiae in vineyards located in South France and North Portugal. This is the first systematical, 3-years biogeographical survey of fermentative S. cerevisiae strains by microsatellite genotyping, aiming at the analysis of populational structures and genetic variability in three vineyards of the Vinho Verde Wine Region of Portugal.

Materials and Methods

Samples

The sampling plan included four vineyards in the North of Portugal (Região Demarcada dos Vinhos Verdes), and South of France (Languedoc) as shown. In each vineyard, six sampling points were defined. Sampling campaigns were performed in duplicate (some days before and some days after harvest). The yeast flora from fermenting grape juice (500 ml) was analysed when the must weight was reduced by 70 g/l, corresponding to the consumption of about 2/3 of the sugar content. This experiment was repeated in three consecutive years (2001-2003), resulting in a total of 83 grape samples and 2490 isolates, that were further analyzed by molecular methods.



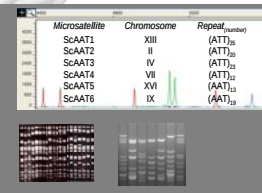
Molecular identification

In France

Preliminary discrimination between Saccharomyces and non-Saccharomyces yeast was based on the inability to grow in YNB medium containing L-lysine [4]. Chromosomal polymorphisms were studied by pulsed field gel electrophoresis as previously described [5]. Strains with identical mtDNA RFLP patterns were grouped and one representative strain was further characterised by analysis of 6 microsatellite loci [6].

In Portugal

Isolated strains were analysed by mitochondrial DNA restriction patterns (mtDNA RFLP) [7]. Strains with identical mtDNA RFLP patterns were grouped and one representative strain was further characterised by analysis of the above mentioned microsatellite loci.The equivalence of this typing method to previously described ones has been previously shown [8]. Microsatellite loci are specific for Saccharomyces cerevisiae. Other Saccharomyces species that may be present in spontaneous fermentations such as S. bayanus and S.paradoxus showed no amplification signals in most of the loci.



Computer assisted data analysis

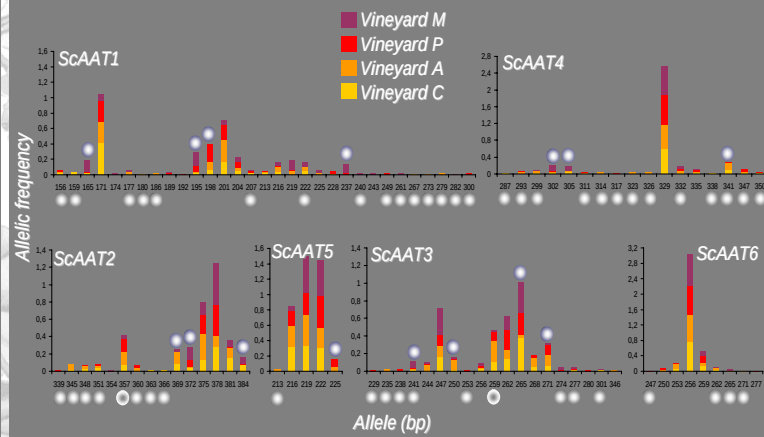
A group of strains with unique microsatellite profiles (obtained from 30 isolates per fermentation) was considered the population corresponding to each sampling site. The pattern and degree of temporal and spatial divergence in the nuclear microsatellites ScAAT1 to ScAAT6 among subpopulations was estimated by Fst determination over all loci by AMOVA analysis (computed by the Arlequin software [9]). A similarity matrix of allelic frequencies was computed by the program NTSYSpc 2.0 [10], based on the Euclidean distance and average linkage (UPGMA).

RESULTS

● Number of perennial genotypes (regional distribution)
① Number of perennial genotypes (limited to one vinayard)
Number of annual genotypes (multiple sites of one vineyard)
Number of annual genotypes (in multiple sites of two vineyards)

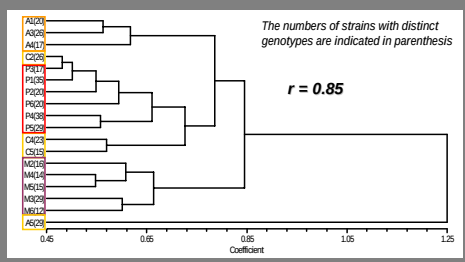
Winery	Year	Number of collected samples	Number of spontaneous fermentations	Number of Isolates	Number of Non-S. cerevisiae isolates	Number of genotypes
A	2001	12	3	90	0	11
	2002	6	6	180	0	34
	2003	12	6	180	0	41
C	2001	12	8	240	0	26
	2002	3	1	30	0	1
	2003	12	7	210	0	35
P	2001	12	8	240	0	64
	2002	9	5	150	0	12
	2003	12	10	300	0	59
M	2001	12	11	330	129	51
	2002	12	12	360	359	1
	2003	12	6	180	59	21

- *The strain collection obtained from this survey comprises 2490 isolates, that were classified in 356 genetic patterns according their allelic distribution.*
- *The highest S. cerevisiae biodiversity was observed in winery M (323 isolates, 73 patterns) followed by wineries P (690 isolates, 135 patterns), A (450 isolates, 86 patterns) and C (480 isolates, 62 patterns).*
- *Several genotypes showed a wider temporal and geographical generalized pattern of sporadic presence, absence and reappearance across sampling sites, vineyards or years.*
- *Non-Saccharomyces strains belonging to the genus* Kloeckera.



- *The six markers revealed a high degree of genetic variability, being ScAAT1 and ScAAT3 the most polymorphic markers with 31 and 19 alleles, respectively.*
- *Besides the 41 ScAAT1-ScAAT6 alleles previously described for 51 strains [3], 52 new alleles were identified in the present study.*
- *Some newly described alleles occur with relative high frequency and may be used as indicative alleles for the Vinho Verde Wine Region.*
- *The vast majority of alleles were evenly distributed among S. cerevisiae populations belonging to vineyards A, C and P and M, but differences are notorious for few alleles, which can be considered as vineyard(s) or Wine Region – indicative.*

Relationships among the populations from different sampling sites in four vineyards



- *Vineyard-specific populational substructure is shown by several clusters, comprising sampling sites of vineyards C, P, A and M. Populations within groups C and P are more closely related, while S. cerevisiae populations belonging to vineyard A are much more heterogeneous and also more distinct from C and P.*
- *The C2 population lies within the P-cluster, indicating that genetic differences do not delimit specific populations with fixed geographic boundaries.*
- *Strains from Languedoc form a cluster that can be clearly distinguished from strains isolated in the Vinho Verde Region*
- *Exceptions from the vineyard - specific population structure may be due to the presence of rare alleles (A5).*

AMOVA analysis - F_{ST} values based on microsatellite data

Source of variation			AG	APWG	WP	F_{ST}	P (r < o)
Among vineyards	2001	A/P	3.03	9.03	87.94	0.12	< 0.0001
	2002		6.38	13.28	80,33	0.20	0.0001
	2003		2.76	11.29	85.95	0.14	0.0001
	2001	A/C	-4.16	16.66	87.51	0.12	0.059
	2003		1.09	16.20	82.71	0.17	< 0.0001
	2001	C/P	-1.21	8.31	92.89	0.07	0.0001
	2003		0.48	8.10	91.42	0.09	< 0.0001
	2001	M/A	5.87	7.33	86.80	0.13	< 0.0001
	2003		7.38	14.59	78.04	0.22	< 0.0001
	2001	M/C	0.03	5.72	94.25	0.06	0.016
	2003		3.85	9.25	86.90	0.13	0.001
	2001	M/P	2.75	5.44	91.80	0.08	< 0.0001
	2003		3.48	4.54	91.98	0.08	< 0.0001
Among years	2001 / 2002	A	-2.45	13.94	88.51	0.11	0.034
		P	0.79	9.94	89.27	0.11	0.0001
	2002 / 2003	A	1.29	15.79	83.0	0.17	< 0.0001
		P	1.68	7.73	90.59	0.09	0.052
	2001 / 2003	A	-2.45	20.48	82.05	0.18	< 0.0001
		C	-1.56	12.67	88.89	0.11	0.0001
		M	-0.25	5.63	94.61	0.05	0.07
		P	0.37	6.30	93.33	0.07	0.0001

WP — within population (group of genotypes from duplicate campaigns of one sampling site);
APWG — among populations within groups of strains from different sampling years / vineyards
AG — among groups of strains from different sampling years / vineyards

- *Genetic distance, expressed as F_{st} values, are not correlated with the distance between vineyards:*
  Pair wise association of populations from different vineyards showed that the closer vineyards A/P and A/C (30 – 50 km) are genetically less related compared to the more distant vineyards P/C (ca. 80 km).
  F_{st} values for the pair wise association of S. cerevisiae populations from France and Portugal (A/M, C/M and P/M) are similar to the values observed among Portuguese populations.
- *Populations variation within a vineyard in consecutive years is similar to the variation observed between vineyards, being more variable in A (Fst = 0.11 – 0.18) compared to P (Fst = 0.09 – 0.11).*

References

[1] D. Schuller, H. Alves, S. Dequin, M. Casal. 2005. FEMS Microbiol Ecol 51, 167-177.
[2] E. Valero, D. Schuller, B. Cambon, M. Casal, S. Dequin. 2005. FEMS Yeast Res. 5, 959-969.
[3] Pretorius IS, du Toit M, van Rensburg P (2003). Food Technol Biotechnol 41:3-10.
[4] Barnett J. A., Payne, R. W., and Yarrow, D. 1990. Yeasts: Characteristics and identification. 2nd Ed. Cambridge University Press. Cambridge. UK.
[5] Blondin, B., and Vezinhet, F. 1988. Rev. Fr. Oenol. 28: 7-11.
[6] Pérez, M.A., Gallego, F.J., Martinez, I. and Hidalgo, P. 2001. Lett. Appl. Microbiol. 33, 461-466.
[7] López V., Querol A., Ramón D., and Fernández-Espinar M.T. 2001. Int J Food Microbiol 68:75-81
[8] Schuller, D., Valero, E., Dequin, S. and Casal, M. 2004. FEMS Microbiol Lett. 231(1) 19-26.
[9] Schneider, S., Kueffer, J-M., Roessli, D. and Excoffier, L. 1997. ARLEQUIN version 1.1. Software for population genetics data analysis. Geneva, Switzerland
[10] NTSYSpc 2.0, 1997, Exeter Software (http://www.ExeterSoftware.com)

Conclusions

Microsatellite typing of loci ScAAT1-ScAAT6, followed by statistical analysis permitted a high resolution populational screen, and is therefore the appropriate method to obtain a deeper insight in the ecology and biogeography of fermentative S. cerevisiae strains, even among geographically close regions.

Genetic differences among S. cerevisiae populations derived mainly from gradations in allele frequencies rather than from distinctive "diagnostic" genotypes, and the accumulation of small allele-frequency differences across six loci allowed the identification of a population structure.

The extension of the current approach to strains isolated from other viticultural regions is currently underway, since a preliminary comparison revealed major differences in both allelic combinations and frequencies (our unpublished data).

Acknowledgements

This study was supported by the projects ENOSAFE (Nº 762, Programa AGRO, medida 8) and the programme POCI 2010 (project POCI/AGR/56771/2004). We appreciate the kind assistance of the enologists Rui Cunha, Anselmo Mendes, Euclides Rodrigues and José Domingues for facilitating sampling campaigns in the three vineyards. Magda Silva Graça is gratefully acknowledged for the operation of the DNA sequencer.

This poster is available at
http://repositorium.sdum.uminho.pt

Dorit Schuller
Centro de Biologia,
Campus de Gualtar
Universidade do Minho
4710-057 Braga, Portugal
Tel: 253 - 60 40 10/17
Fax: 253 – 67 89 80
dschuller@bio.uminho.pt